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Regulation of Light Output and Identification of Luminous Bacteria from free-living and particle-associated bacterial Assemblages

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13. ABSTRACT (Maximum 200 words)

Samples from a culture of the marine nitrogen-fixing bacteria Vibrio natriegens were collected from aerobic/combined-nitrogen replete conditions and ar regular intervals after shifting the culture to anaerobic/combined-nitrogen absat conditions. RNA and proteins were extracted from the samples for filter-binding assays using a 1.9 kb nifHD probe and antisera to nitrogenase. Results indicated that nifH mRNA and nitrogenase began to appear 100-120 min after shifting the culture from aerobic/combined-nitrogen replete to anaerobic/combined-nitrogen absent conditions. After 6-9 h (early log growth) nifH mRNA disappeared, whereas nitrogenase remained throughout log growth up to 18 h after shift. Additionally, both nifH mRNA and nitrogenase disappeared within 40 min after shifting the culture from nitrogen-fixing to non-nitrogen-fixing conditions; nifH mRNA disappeared within 10 min after the shift in a subsequent experiment

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Nitrogenase, nitrogen-fixing, Vibrio natriegens, marine bacteria

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ANNUAL REPORT

Grant # N00014-91-J-1885 R&T Code = 441p006

PRINCIPAL INVESTIGATOR: Dr. James A. Coyer

INSTITUTION: University of California-Los Angeles

GRANT TITLE: Regulation of Light Output and Identification of Luminous Bacteria From Free-living and particle-associated Bacterial Assemblages

REPORTING PERIOD: 1 January 1993 - 31 December 1993

AWARD PERIOD: 1 January 1993 - 31 December 1993

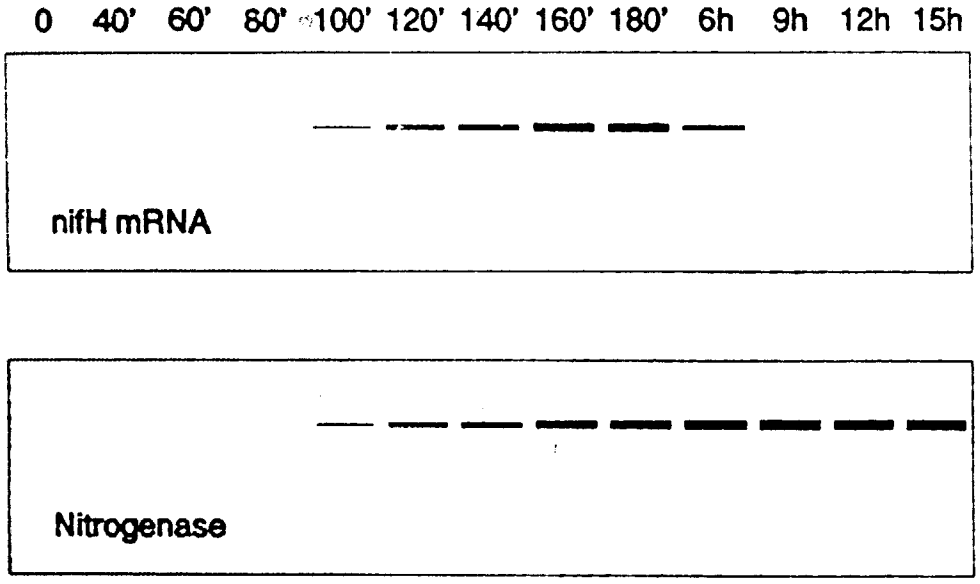
OBJECTIVE: To determine how changes in environmental conditions affect gene transcription and translation.

APPROACH: Samples from a culture of the marine nitrogen-fixing bacteria Vibrio natriegens were collected from aerobic/combined-nitrogen present conditions and at regular intervals (40 min-15 hrs) after shifting the culture to anaerobic/combined-nitrogen absent conditions. RNA and proteins were extracted from the samples for filter-binding assays (northerns, westerns) using a 1.9 kb nifHD probe and antisera to nitrogenase. In situ hybridization techniques are used to visualize those cells from a population of cells that are potential nitrogen-fixers or are actively fixing nitrogen. Primers were designed to amplify (via the polymerase chain reaction) a 296 bp nifH segment from the 1.9 kb nifHD insert excised from the plasmid pSA30 using restriction enzymes EcoRI and BamHI.

ACCOMPLISHMENTS (last six months): Results of the shift experiments indicate that nifH mRNA and nitrogenase begin to appear 100-120 minutes after shifting the culture from aerobic/combined-nitrogen present to anaerobic/combined-nitrogen absent conditions. After 6-9 hrs (early log growth), nifH mRNA disappeared, whereas nitrogenase remained throughout log growth up to 18 hrs after shift. Additionally, both nifH mRNA and nitrogenase disappeared within 40 minutes after shifting the culture from anaerobic (nitrogen-fixing) to aerobic (non-nitrogen-fixing) conditions; nifH mRNA disappeared within 10 minutes after the shift in a subsequent experiment. The hybridization pattern to RNA from nitrogen-fixing bacteria using the PCR-derived 296 bp probe to a conserved portion of nifH is identical to the hybridization pattern revealed with a 1.9 kb probe complementary to nifH and partial nifD.

SIGNIFICANCE: Marine nitrogen-fixing bacteria respond rapidly to anaerobic, low combined nitrogen conditions by simultaneously transcribing and translating nif genes to nitrogenase. However, the mRNA is soon degraded, whereas the nitrogenase remains active for many hours. In situ hybridization techniques, therefore, must utilize both nucleic acid probes as well as antisera to nitrogenase when resolving nitrogen-fixing species from a mixed population of bacteria.

WORK PLAN (next 6 months): The specific objective of the next work period is to refine the 296 bp probe for in situ hybridizations. One approach is to label the 296 bp probe with digoxigenin (DIG) during PCR amplification. After in situ hybridization to nifH, the DIG-labeled probe can be detected with fluorescently-labeled anti-DIG using epifluorescence microscopy. Another approach is to use the PCR primers, nifH mRNA from a bacterial culture harvested during nitrogen-fixation, and reverse transcriptase to produce a cDNA probe complementary to the 296 bp conserved region of nifH. A cDNA probe will increase the efficiency of hybridization and signal strength.



OBJECTIVES

- Determine time course of nifH mRNA after shift to Nitrogen-fixing conditions
- Determine time course of nitrogenase after shift to Nitrogen-fixing conditions
- Identify Nitrogen-fixing bacteria with DNA probe to nif gene

ACCOMPLISHMENTS

- nifH mRNA appears 100-120 minutes and disappears 6-9 hrs after shift to Nitrogen-fixing conditions
- Nitrogenase appears 100-120 minutes and remains through 18 hrs after shift to Nitrogen-fixing conditions
- 296 bp probe to nifH generated by PCR and binds to nifH mRNA extracted from Nitrogen-fixing bacteria

SIGNIFICANCE

- Marine Nitrogen-fixing bacteria respond rapidly to Nitrogen-fixing conditions
- In situ hybridization techniques must use both nucleic acid probes and antisera to resolve Nitrogen-fixing bacteria from mixed populations